

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets

(11) Publication number:

0 103 081
A2

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 83105982.9

 (51) Int. Cl.³: **A 61 D 7/00, A 61 M 25/00,**
A 61 M 1/00

(22) Date of filing: 18.06.83

 (30) Priority: 30.03.83 US 480355
 06.07.82 US 395830

 (71) Applicant: Cullor, James S., 128 Shasta Place, Woodland
 Calif. 95615 (US)
 Applicant: Smith, Wilbur D., Rt. 3, Nevada
 Missouri 64772 (US)
 Applicant: Cullor, James Sr., Rt. 1, Box 62A, Fort Scott
 Kansas 66701 (US)
 Applicant: Cullor, Gary W., Rt. 5, Fort Scott
 Kansas 6701 (US)

 (43) Date of publication of application: 21.03.84
 Bulletin 84/12

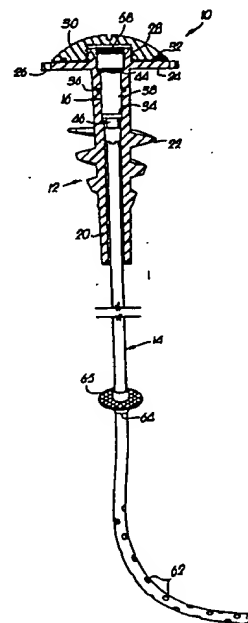
 (72) Inventor: Cullor, James S., 128 Shasta Place, Woodland
 Calif. 95615 (US)

 (84) Designated Contracting States: AT BE CH DE FR GB IT
 LI LU NL SE

 (74) Representative: UEXKÜLL & STOLBERG Patentanwälte,
 Beselerstrasse 4, D-2000 Hamburg 52 (DE)

(54) Method and apparatus for monitoring body parts of animals.

(57) An improved method and apparatus is disclosed for adding fluids to, or removing fluids from, a body part or organ of an animal which minimizes animal trauma and permits rapid, easy, repeated fluid or low viscosity gel transfers. The apparatus includes a tubular fluid-conveying element adapted for fixed connection to the animal, along with a flexible fluid conduit connected between the element and a specific internal body part or organ, such as a cow's uterus. In use, a syringe is employed to introduce or remove fluids from the body part, through the tubular element and connected conduit. The invention is especially adapted for introducing and recovering cell lines producing monoclonal antibodies or other biologically active products in large mammals, and facilitates monitoring of antibody production as well as administration of nutrients to enhance cell line growth. In preferred monoclonal antibody production procedures, the cells are initially educated through in vitro contact with ambient fluid of the selected organ (e.g., allantoic fluid from a pregnant host), whereupon the educated cells are inoculated and allowed to incubate in the host. It has been found particularly advantageous to employ the allantois of a pregnant mammal for antibody production, and some or all of the cell incubation period therein should occur after the onset of immunocompetency of the fetus.



1 METHOD AND APPARATUS FOR MONITORING BODY PARTS
 OF ANIMALS

5 This is a continuation-in-part of identically titled application for U. S. Letters Patent Serial No. 06/395,830, filed July 6, 1982.

Background of the invention

10 1. Field of the Invention

 The present invention is broadly concerned with a method and apparatus designed to greatly facilitate addition of fluids to, or removal of fluids from, an internal body organ or part of an animal.
15 More particularly, it is concerned with such a method and apparatus which is especially suited for the large scale production of monoclonal antibodies in large mammals, and which permits repeated monitoring and/or nutritional enhancement of the in vivo monoclonal antibody production procedure. In preferred
20 methods, monoclonal antibody production is enhanced by in vitro education of the cells using fluid characteristic of the host organ; moreover, use of the allantois of a pregnant host (e.g., cow, horse, sheep
25 or pig) after the onset of fetus immunocompetency is especially advantageous.

 2. Description of the Prior Art

 When a foreign substance enters the body of a vertebrate animal or is injected into it, one
30 aspect of the immune response is the secretion by plasma cells of antibodies. Quite apart from the natural function of antibodies in the animal's immune response, such antibodies have long been an important tool for investigators, who capitalize on their
35 specificity to identify or label particular molecules

1 or cells and to separate them from a mixture. The
antibody response to a typical antigen is normally
highly heterogeneous, and even the best of antisera
are really heterogeneous mixtures of many different
5 antibody molecules that vary in charge, size, and in
such biologic properties as the ability to fix com-
plement or to agglutinate or precipitate antigen. It
is extremely difficult to separate the various anti-
bodies in antisera, and therefore conventional anti-
10 sera contain mixtures of antibodies, and such mix-
tures vary from animal to animal.

It is also known that malignant tumors of
the immune system (called myelomas) are characterized
by rapidly proliferating cells producing large amount
15 of abnormal immunoglobulines called myeloma proteins.
A tumor itself is considered to be an immortal clone
of cells descended from a single progenitor, and so
myeloma cells can be cultured indefinitely, and all
the immunoglobulins they secrete are identical in
20 chemical structure. They are in effect monoclonal
antibodies, but there is no way to know what antigen
they are directed against, nor can one induce myel-
omas that produce antibody to a specific antigen.
However, in recent years researchers have learned how
25 to fuse myeloma cells of mice with lymphocytes from
the spleen of mice immunized with a particular anti-
gen. The resulting hybrid-myeloma, or "hybridoma"
cells express both the lymphocyte's property of
specific antibody production and the immortal char-
acter of the myeloma cells. Such hybrid cells can be
30 manipulated by the techniques applicable to animal
cells in permanent culture. Individual hybrid cells
can be cloned, and each clone produces large amounts
of identical antibody to the single antigenic deter-
35 minant. The individual clones can be maintained

1 indefinitely, and at any time samples can be grown in
culture or injected into animals for large scale
production of monoclonal antibody. Highly specific
monoclonal antibodies produced by this general method
5 have proved to be a versatile tool in many areas of
biological research and clinical medicine.

While the utility of specific monoclonal
antibodies is manifest, a problem has arisen because
of the difficulty of producing significant (e.g.,
10 liter) quantities of the antibodies. Obviously, the
production of such antibodies in mice is not at all
suited for large scale production.

In response to this problem, it has been
suggested to employ large mammals such as cattle or
15 sheep for in vivo production of monoclonal anti-
bodies. In one such procedure, the cells of hybri-
doma clones are introduced into the amniotic fluid of
a cow in the early stages of gestation prior to the
onset of fetal immunocompetency and are allowed to
20 multiply. After a suitable growth period, quantities
of monoclonal antibodies can be harvested. While the
above described technique shows considerable promise,
a number of practical problems remain. For example,
it is desirable to monitor the production of mono-
25 clonal antibodies during incubation thereof, and the
problems of obtaining samples of the amniotic fluid
on a frequent recurrent basis are formidable. The
straightforward procedure of simply making a lapar-
otomy incision in the cow's body, manipulation of the
30 uterus, introducing or withdrawing materials, can
create multiple insults to the cow, uterus and fetus,
which may lead to premature death, infection, or
abortion of the fetus. By the same token, in order
to enhance antibody production in the amniotic fluid,
35 it is oftentimes desirable to introduce nutrient

1 fluids into the uterus. Here again, the conventional
techniques for such introduction, if used repeatedly,
can cause severe problems to the animal and uterine
environment.

5 Prior art patents describing various types
of percutaneous transport tubes and related structure
include: U.S. Patents Nos. 4,315,513, 3,401,689,
3,515,124, 3,570,484, 3,583,387, 3,961,632 and
3,333,588. All of the structures described in the
10 aforementioned patents are deficient in important
respects, and would present serious problems if it
were attempted to use the same in the context of in
vivo monoclonal antibody production.

15 In addition, the prior practice of simply
placing a cell line in amniotic fluid often leads to
rapid death of a large proportion of cells. That is
to say, cell lines of interest are typically cultured
in highly specific and optimized media, and under
relative critical conditions. For example, many cell
20 lines are cultured and allowed to multiply at incuba-
tion temperatures of 37 degrees centigrade in speci-
ally prepared media, with periodic screening and
feeding at regular intervals (e.g., every 48-72
hours). Prior to inoculation, the cells are concen-
25 trated into a fixed volume of their media plus fetal
calf serum and are surgically implanted. However,
this procedure gives only mediocre results, and can
often fail completely in that the cells do not multi-
ply or do not produce the desired antibody. One
30 problem with this approach is that the cells, when
inoculated, can experience severe "shock" owing to
the radically different ambient environment of the
amniotic fluid as compared with the previous in vitro
culture media and scrupulously maintained growth
35 conditions.

1 Finally, use of amnionic fluid of a preg-
nant host as a growth media for hybridoma cells leads
to a number of additional difficulties. Specific-
ally, in the case of cattle, it is known during the
5 second trimester of gestation the amnionic fluid
consistency changes and it becomes gel-like. More-
over, the fetus swallows substantial quantities of
the amniotic fluid, especially during the first
trimester of gestation. For the foregoing reasons,
10 prior in vivo attempts to produce monoclonal anti-
bodies using amnionic fluid have been conduted during
early stages of gestation so that the entire proce-
dure is accomplished prior to the onset of fetal
immunocompetency. If the procedure is carried on
15 after the fetus becomes immunocompetent, the fetus
will treat the inoculated cells as antigens and will
develop appropriate antibodies, thereby killing the
cells. Accordingly, the gestational time frame for
cell growth is severely limited and critical in the
20 case of amnionic fluid. Also, surgical manipulation
of the fetus and amnionic sac during the early stages
of pregnancy is difficult (because of uterine anatom-
ical positioning, flank incisions, high paralumbar
fossa, must be utilized), and this can lead to spon-
25 taneous abortion and/or fetal infection. Thus, while
use of the amnionic fluid of a pregnant host is
theoretically possible, a number of practical prob-
lems remain.

It will therefore be seen that there is a
30 real and unsatisfied need in the art for methods and
apparatus which permit easy, rapid addition to fluids
to, or removal of fluids from, specific body parts or
organs of animals, so as to facilitate the production
and monitoring of moniclonal antibodies, while at the
35 same time avoiding repeated insults to the animal and

1 other internal organs and problems of unintended cell
death and low levels of antibody production.

Summary of the Invention

5 The foregoing problems are in large measure
solved by the present invention which provides, in
one aspect, a greatly improved method and apparatus
especially adapted for the large scale cell growth in
10 living mammals, such as in the production of mono-
clonal antibodies. Broadly speaking, the apparatus
is designed for selectively withdrawing fluids from,
or adding fluids to, an animal's body, and includes
an assembly including a tubular, fluid-conveying
15 element designed to be coupled to the animal's body
with the outer end of the element being adjacent the
exterior surface of animal's skin. An elongated,
flexible, fluid-conveying conduit having an inner end
and an outer end also forms a part of the apparatus;
20 the outer end of the conduit is operably coupled and
in fluid flow communication with the tubular element,
whereas the inner conduit end is configured for
insertion through the wall of a specific organ or the
like of the living animal, with the innermost portion
25 of the flexible conduit lying within the organ. The
conduit is advantageously of a length greater than
the shortest distance between the element and the
point of insertion thereof through the wall of the
organ so that the conduit permits and accommodates
30 natural movement of the organ over time. Finally,
the overall apparatus includes means for physically
attaching and interlocking the inner end of the
conduit to the organ wall so as to prevent leakage of
fluid from the organ.

35 In preferred forms, the overall fluid-
conveying element includes selectively operable valve

1 means which minimizes the risk of infection of the
host animal; however, such valve apparatus is not
absolutely essential.

5 In other forms of the apparatus, the inner-
most portion of the conduit is of a larger diameter
than the remaining portion of the conduit, and is
formed of a soft, flexible material such as silicone
rubber.

10 In the use of the preferred apparatus of
the invention, the fluid-conveying element is secured
to the animal's body with the outer end of the ele-
ment adjacent the exterior surface of the animal's
skin, and the fluid conduit is coupled to the element
and a selected organ such as a uterus. The coupling
15 step involves making an incision in the wall of the
selected organ and inserting the inner end of the
conduit into the organ. The conduit is physically
attached and interlocked to the wall of the organ, as
by suturing an appropriate collar affixed to the
20 conduit, so that leakage from the organ is prevented.
Once the apparatus is installed, fluid can be peri-
odically and selectively withdrawn from, or added to,
the organ, as by use of a conventional syringe or the
like.

25 In other aspects of the invention, it has
been found particularly advantageous to "educate"
cells prior to inoculation thereof into the host
organ. Such education generally includes contacting
the cells with ambient fluid characteristic of the
30 selected host organ or the like, on an in vitro
basis. Typically, the cells are repeatedly contacted
with respective quantities of the ambient fluid over
a period of time, with the concentration of the
ambient fluid being increased. In the case of bovine
35 allantoic fluid, the initial contact would be with a

1 fluid containing from about 5 to 10 percent by volume
of the allantoic fluid, followed by step-wise con-
tacting of the cells with additional fluid samples
5 containing increasing concentrations of the allantoic
fluid. During this education procedure, the cells
become acclimated to the allantoic fluid and begin to
multiply therein.

It has also been found that an improved
method for cell growth can be provided by inoculating
10 cells into the allantois of a pregnant host, parti-
cularly an animal selected from the group consisting
of sheep, horses, pigs and cows. The cells are
caused to multiply in the allantois of the host over
a period of time, with at least a portion of the time
15 period being after the onset of immunocompetency of
the host's fetus. In particularly preferred forms,
the entire procedure occurs after the onset of fetus
immunocompetency, typically during the second and
third trimesters of gestation. The final step in-
20 volves harvesting the cells or the secretory products
thereof, e.g., monoclonal antibodies.

Brief Description of the Drawings

Figure 1 is a fragmentary view in partial
25 vertical section illustrating one embodiment of a
fluid-conveying apparatus in accordance with the
present invention;

Fig. 2 is an enlarged, fragmentary view in
partial vertical section illustrating the valve as-
30 sembly of the Fig. 1 apparatus, with a syringe posi-
tioned for operating the internal valve means there-
of;

Fig. 3 is a side elevational view of the
valve assembly prior to application thereof to an
35 animal;

1 Fig. 4 is a top view of the assembly depicted in Fig. 3;

5 Fig. 5 is a top view, with the uppermost protective cap removed, of the assembly illustrated in Fig. 3;

 Fig. 6 is a view similar to that of Fig. 5, but illustrates the inner protective cap removed to expose the internal valve means;

10 Fig. 7 is a schematic, sectional view illustrating the abdominal cavity of a cow, along with the cow's uterus, and with the valve assembly of the Fig. 1 embodiment inserted in place on the cow extending into the cow's body;

15 Fig. 8 is a view similar to that of Fig. 7 and illustrates the uterus removed with the inner end of the fluid-conveying conduit secured to the uterine wall;

20 Fig. 9 is a view similar to that of Fig. 8, but illustrates the complete Fig. 1 apparatus in place;

25 Fig. 10 is a view similar to that of Fig. 1 but illustrating another embodiment of the invention wherein use is made of a fluid-conveying conduit with the inner portion thereof having a diameter larger than that of the main portion of the conduit;

 Fig. 11 is an enlarged, fragmentary view in partial vertical section illustrating the valve assembly of the Fig. 1 apparatus, with a syringe positioned for operating the internal valve;

30 Fig. 12 is a view similar to that of Fig. 11 but illustrating another embodiment which does not employ an internal, mechanical valve; and

 Fig. 13 is a fragmentary view in partial section depicting a fluid-conveying conduit for use

- 1 in the invention wherein the conduit includes a pair of telescopically interfitted tubular members.

Description of the Preferred Embodiments

- 5 Turning now to the drawings, and particularly Figs. 1-6, conveying apparatus 10 in accordance with the invention is illustrated. Broadly speaking, the apparatus 10 includes a valve assembly 12 along with an elongated conduit 14. The valve assembly 12
10 in turn includes an elongated, tubular, fluid-conveying element 16, as well as valving means 18 carried within the element 16.

- In more detail, the valve assembly 12 preferably includes an elongated, slightly tapered, open ended outer tubular component 20 formed of an
15 appropriate synthetic resin material and having an outwardly extending screw thread 22 formed thereon. The upper end of the component 20 is provided with an enlarged flange-type head 24 having a plurality of
20 circumferentially spaced apertures 26 therethrough, as well as an upstanding, central, threaded annular connector 28. An outermost, rounded, removable synthetic resin cap 30 is threaded onto connector 28 as illustrated, and includes an O-ring seal 32 which
25 abuts the upper surface of head 24 (see Fig. 1). The component 20 is configured to present a continuous, stepped, internal bore 34 along the length thereof and has an internal O-ring seal 36 spaced downwardly from head 24.

- 30 The tubular element 16 is situated within the upper, enlarged diameter portion of bore 34, and includes a synthetic resin tubular member 38 which is threaded at its upper end, the latter extending to a point adjacent surrounding connector 28. The inner
35 surface of member 38 defines a fluid-conveying pass-

1 age 40, and is configured to present an annular,
obliquely oriented engagement surface 42 which is
important for purposes to be made clear. Another
5 seal 44 is disposed about the upper end of the member
38, and is situated within the upper end of bore 34
(see Fig. 2).

The tubular element 16 also includes a
lowermost tubular part 46 which is received within
the lower end of member 38 and presents an annular
10 abutment surface 48. The lower end of part 46 is of
frustoconical configuration as best seen in Fig. 2.
In addition, it will be observed that the bore of
part 46 is coaxial and in communication with the
passage 40 of member 38.

15 Valving means 18 is situated to normally
block flow of fluid through the element 16. In
detail, the valving means 18 includes a shiftable
plug 50 presenting a conical upper sealing surface 52
which is complementary with and adjacent engagement
20 surface 42 of member 38. A helical spring 54 is
situated between the underside of plug 50 and the
abutment surface 48, and serves to bias plug 50
against engagement surface 42 for purposes of nor-
mally sealing the member 38, and thus overall tubular
25 element 16, against fluid flow therethrough.

A tubular actuator 56 is positioned atop
plug 52 and in engagement with the latter. The
actuator 56 extends upwardly from the plug 50 and
into the annular region defined by the threaded upper
30 end of the member 38. It will be observed in this
respect that the actuator 56 is centrally bored for
passage of fluids.

Referring to Figs. 1 and 5, it will be seen
that an inner cap 58 is advantageously applied to the
35 upper threaded end of member 38, in order to further

1 seal the internal valving means 18 when the latter is
not in use. In addition (see Fig. 3), a removable
insertion tip 60 is affixed to the lower open end of
the tubular component 20.

5 The conduit 14 is advantageously in the
form of plastic tubing which is dimensioned to re-
ceive and tightly engage the lower end of part 46
(see Figs. 1 and 2), and thus be operatively con-
nected to the tubular element 16. The end of conduit
10 14 remote from the valve assembly 12 is provided with
a plurality of fluid flow apertures 62 through the
defining wall thereof. In addition, an attachment
collar 64 is situated on conduit 14 intermediate the
ends thereof, and has an annular, outwardly extending
15 cloth or synthetic resin attachment flange 65. As
shown, this flange 66 is flexible and perforate and
can be sutured to an organ wall; the flange should
therefore extend at least about one-quarter of an
inch outwardly from the conduit. The innermost
20 portion of conduit 14 (i.e., the fenestrated portion
below the flange 65) is adapted for insertion into the
confines of the animal's organ. In Figures 7-9, the
abdominal cavity 66 of a cow 68 is shown. The uterus
70, supported by ligaments 72, 74 is likewise de-
25 picted.

Installation of device 10 on cow 68 may
involve initially tranquilizing the cow and admini-
stering a local anesthesia at the selected laparotomy
site(s), typically left or right paralumbar fossa. A
30 skin incision is next made, typically a caudal and
dorsal to laparotomy incision. The valve assembly 12
is next inserted into the incision using a rotating
action so that the component 20 is in effect screwed
into and through the abdominal cavity wall until the
35 inner end of the valve assembly is disposed within

1 cavity 66 (see Fig. 7). Tack down sutures of non-
absorbable suture material are next installed through
the apertures 26 in head 24, in order to fixedly
position the component 20, and thereby the element 16
5 and valving means 18 carried therein, on the cow 68.

The cow's uterus is next grasped and pulled
outside of cavity 66 (see Fig. 8). If necessary,
another incision is made in the abdominal cavity wall
to permit such manipulation of the uterus. In any
10 event, one may choose to make a small (1 cm.) incision
through the uterine wall, and the fenestrated
conduit 14 is inserted into the uterus; sufficient
length is allowed within the uterus for uterine
growth and descent into the abdominal cavity as
15 gestation proceeds. That is to say, the length of
conduit 14 is greater than the shortest distance
between the element 16 and the point of insertion
through the uterus; this construction along with the
flexibility of the conduit, permits and accomodates
20 natural movement of the uterus over time. A so-
called "purse string" suture is then placed through
the uterine wall and the flange 65, using suture
material. The incision and suture are then checked
for fluid leakage, and the collar 64 is further
25 secured to conduit 14 by gluing. In this fashion,
the inner end of conduit 14 is physically attached
and interlocked with the uterine wall; and this
effect is enhanced by virtue of the formations of
adhesions around the suture site which occurs within
30 a few days after device 10 is installed.

The uterus 70 is next replaced in its
normal position, making certain that sufficient
excess tubing is present to connect with the part 46
and allow for normal animal movement and fetal
35 growth. The penetrating point or tip 60 is next

1 removed, and the free end of conduit 14 is opera-
tively coupled with the valve assembly 12 by passing
the end of the conduit into component 20 and over
part 46 (see Fig. 2). The caps 30 and 58 are then
5 removed, and a syringe 76, with needle removed, is
used to aspirate the apparatus and check for fluid
flow. The caps 30, 58 are next replaced, and the
laparotomy incision is closed.

10 In a typical procedure for the production
of monoclonal antibodies, inoculation of the cow's
uterus or fetus may occur 5-7 days after installation
of apparatus 10, assuming that the cow's systemic
inflammatory response has decreased and after it has
15 been determined that the pregnancy is being main-
tained. Such inoculation would include introduction
of conditioning reagents (e.g., pristane, albumins
and the like) in uterine fluids, followed by inocula-
tion of the cell lines. Incubation varies with the
specific cell line selected, and in general the
20 uterine fluids are monitored periodically using
apparatus 10. When it is desired to harvest the cell
line and its products, such may be accomplished
through the use of device 10, through cesarean sec-
tion, or by sacrificing the cow and obtaining the
25 selected utrine fluids. Typical enrichment consti-
tuents added to the uterine fluid during the incuba-
tion sequence would include, amino acids, bovine
serum albumin, vitamins, inorganic salts, and suspen-
sion mediums and growth factors. More specifically,
30 amino acids such as L-Glutamine, L-Argine, L-Cystine,
and L-Histadine $\text{HCl} \cdot \text{H}_2\text{O}$ may be added. Vitamin addi-
tion may include D-Calcium Pantothenate, Thiamine
 HCl , Choline Chloride and Riboflavin. Inorganic
salts may include KCl , NaHCO_3 , $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, and
35 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. Finally, suspension mediums such as

1 Dextrose, Phenol Red, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, and NaCl, KCl may
be included.

5 When it is desired to utilize apparatus 10
either for introduction of fluids into, or removal of
fluids from, the cow's uterus, the following proce-
dure obtains. First, the caps 30, 58 are removed,
and a syringe 76 (see Fig. 2), with needle removed,
is pressed downwardly into the upper end of member 38
until the actuator 56 is encountered. The syringe is
10 thereupon pressed inwardly with the effect that the
plug 50, and particularly surface 52 thereof, is
shifted away from mating surface 42 against the bias
of spring 54. When this occurs, it will be seen that
a fluid flow path is established through tubular
15 actuator 56, passage 40, the bore of part 46, and
conduit 14. Thus, fluids can be administered through
apparatus 10 into uterus 70 simply by manipulating
syringe 76 in the usual injection manner. By the
same token, fluids can be withdrawn from the uterus
20 by the opposite manipulation of syringe 76, as those
skilled in the art will readily appreciate.

It should also be understood that while the
apparatus and method have been illustrated in connec-
tion with a cow's uterus, the invention is not so
25 limited. For example, the apparatus can be used with
virtually any large mammal such as sheep, goats or
cattle. In addition, other body parts or organs can
be connected to the apparatus hereof., e.g., the
bladder, intestine or rumen compartments.

30 In this connection, it is particularly
advantageous to employ pregnant hosts where the
placenta acts as a barrier and will not allow mater-
nal antibodies to cross into the uterine compart-
ments. These types of placenta are sometimes refer-
35 red to as epitheliochorial (found in animals such as

1 horse, pig, cow and sheep) and syndesmochorical.
These placenta provide a functional and immunological
isolation of the fetus from the maternal immune
system, and therefore use of pregnant hoss having
5 these types of placenta is preferred.

Turning now to Figs. 10-11, a second embodiment
of the apparatus is illustrated. In this
instance fluid conveying apparatus 110 is provided
which in many respects is identical to apparatus 10.
10 Thus, the apparatus 110 includes a valve assembly 112
along with an elongated, flexible, fluid conveying
conduit 114. The valve assembly 112 has an elongated,
tubular, fluid-conveying element 116, as well
as valving means 118 carried within the element 116.

15 The overall valve assembly 112 includes an
elongated, tapered, tubular, externally threaded synthetic
resin component 120 having a screw thread 122 and an
enlarged flange-type head 124 having apertures 126
therethrough. The head 124 further includes an
20 upstanding, central, threaded annular connector 128.
Outermost cap 130 is threaded onto connector 128 as
illustrated, and includes an O-ring seal 132 which
abuts the upper surface of head 124. The component
120 presents a continuous, stepped internal bore 134
25 along the length thereof and has an internal O-ring
seal 136 spaced downwardly from head 124.

The tubular element 116 is situated within
bore 34 and includes an upper synthetic resin tubular
member 138 which is threaded at its upper end, the
30 latter extending to a point adjacent the surrounding
connector 128 and having a removable cup 158. The
inner surface of member 138 defines a fluid-conveying
passage 140 (see Fig. 11) and presents an annular,
obliquely oriented engagement surface 142 proximal to
35 the upper end thereof. A secondary O-ring seal 144

- 17.

1 is disposed about the upper end of member 138, and is situated within the upper end of bore 134.

5 The lowermost end of element 116 is internally threaded and receives a lowermost tubular part 146. The part 146 includes an internal bore 147, and extends along the length of bore 134 in substantially conforming relationship thereto. The lowermost end of part 146 is threaded (see Fig. 11), in order to facilitate secure attachment to the outer end of conduit 114 thereto.

10 Valving means 118 is situated to normally block the flow of fluid through the element 116. In detail, the valving means 118 includes a shiftable plug 150 presenting a conical upper sealing surface 152 which is complementary with and adjacent engagement surface 142. A spring 154 is situated between the underside of plug 150 and the uppermost annular surface of part 146, and serves to bias the plug 150 against surface 142 for purposes of normally sealing the member 138, and thus the overall tubular element 116, against fluid flow therethrough.

15 The conduit 14 is advantageously in the form of an outermost stretch of relatively small diameter synthetic resin tubing (e.g., Tygon) 114a which is secured to the part 146, along with an innermost end stretch 114b which is attached to the section 114a. The innermost portion 114b is designed to be inserted through the organ or the like of a host animal, and is advantageously of a somewhat larger diameter than the stretch 114a. The innermost portion 114b includes a series of openings 162 there-through, and is advantageously formed from a soft, flexible material such as silicon rubber.

20 The overall length of the conduit 114 is sufficient to extend between the element 116 and the

1 specific organ of the host animal, with added length
to accommodate and permit natural movement of the
organ.

5 The conduit 114 further includes an attachment
collar 164 which is affixed to stretch 114 adjacent
the innermost end thereof. The collar 164 includes
an outwardly extending flexible flange 166 which can
be physically attached, as by suturing and/or gluing,
to the wall of the selected host
10 organ.

Use of the two component conduit 114 is
advantageous in that the relatively small diameter
stretch 114a resists collapse under suction conditions,
but has sufficient strength to accommodate
15 organ movement without tearing. On the other hand,
the enlarged diameter (up to about one-half inch)
fenestrated portion 114b permits collection of fluid
samples from the organ without clogging or the like.

The device 110 is installed in the same
20 manner as heretofore described with respect to device
10.

During use of apparatus 110 either for
introduction of fluids into, or removal of fluids
from, a host organ, the caps 130, 158 are removed,
25 and a syringe 176, with needle removed, is provided.
A separate, removable, synthetic resin tubular tip
180 is provided having a lowermost, notched plug-
engaging end 182 and is affixed to the delivery end
of flange 176. The flange and tip are then pressed
30 downwardly into the upper end of member 138 (see Fig.
11) in order to shift the plug, and particularly
surface 152 thereof, away from mating surface 142
against the bias of spring 154. When this occurs, a
fluid flow path is established through conduit 114,
35 element 116, tip 180 and syringe 176. Thus, fluids

1 can be administered through apparatus into an organ
simply by manipulating syringe 176 in the usual
injection manner. By the same token, fluids can be
5 withdrawn by the opposite manipulation of syringe
176.

While the apparatus depicted in Figs. 1-6,
and in Figs. 10-11, include a selectively openable
valve assembly 18, 118, the invention is not so limited.
For example, and referring to Fig. 12, an apparatus
10 210 is depicted. This apparatus includes an
outer tubular component 220 identical to the component
120, along with a tubular, capped insert 222.
The insert 222 includes an elongated stepped bore 224
along the length thereof, and is provided with a pair
15 of threaded regions 226, 228 adjacent the lower end
thereof. The component 222 has a threaded uppermost
end 230 and a removable cap 232. The overall apparatus
210 includes a flexible conduit 214 having overall length
and flexibility properties similar to
20 the conduits 14, 114 described above. However, in
this instance a pair of telescopically interfitted
tubular members 234, 236 are employed, with the
innermost 234 being frictionally secured to the
threaded region 228, while the outer member 236 is
25 frictionally coupled to the threaded region 226 (see
Fig. 12). Use of a pair of telescopically interfitted
members can be advantageous, particularly
inasmuch as this construction resists crimping and
resultant fluid blockage during normal movement of
30 the host and/or the selected organ. The innermost
end of the conduit 214 is fenestrated (see Fig. 13),
and includes an attachment collar (not shown).
Installation of device 210 involves the identical
steps depicted and described above.

1 In the use of apparatus 210, the cap 232 is
removed, and a syringe is employed to inject fluid
into, or withdraw fluids from, the host's organ.
However, as mentioned above, this embodiment of the
5 invention does not include a separate valving unit.

As noted above, the apparatus and method of
the invention are particularly adapted for use in
large-scale in vivo production of monoclonal anti-
bodies. To this end, it has been discovered that the
10 cell line to be employed should be initially educated
to the anticipated in vivo environment. Such educa-
tion generally refers to an adjustment of the growth
and metabolism characteristics of the cells to the
new environment, and is generally accomplished by an
15 in vitro contact of the cells with the ambient fluid
to be experienced in the animal host, followed by
inoculation of the educated cells.

In practice, the cell education technique
normally involves repeated or stepwise contact of the
20 cells with respective, increasing quantities of the
ambient fluid over a period of time, typically as
long as several days to several weeks. The ambient
fluid can be derived from the host itself, or more
usually from the same type of animal as the host.

25 In particularly preferred forms, the host
animal is a pregnant cow, and the ambient fluid is
either the allantoic or amniotic fluid of the cow's
uterus, with the allantoic fluid being the most pre-
ferred for reasons hereinafter described. In this
30 case, the cells are contacted with a mixture of
various nutrients and the appropriate uterine fluid,
until cell acclimation is established by the appro-
priate in vitro growth rate. During such in vitro
cell education, the cells are advantageously main-
35 tained at a temperature of from about 38.3-39.0

1 degrees centigrade. In addition, the cells are
initially contacted with a fluid containing from
about 5 to 10 percent by volume of the ambient fluid,
followed by cultivation and incubation therein.
5 Thereafter, the cells may be contacted with a fluid
in increasing concentrations to achieve optimal
growth and education.

In practice, this portion of the technique
is carried out to accomplish the following goals:

- 10 1. To determine if the particular cell line in
question encounters any toxic effects due
to some unknown factor in the ambient
fluid. Such would be indicated by immedi-
ate death or eventual starvation because of
15 some nutrient deficiency;
2. The concentration of ambient fluid at which
slowing or cessation of cell growth may
occur. This is determined by adding the
ambient (e.g., uterine) fluid to normal in
20 vitro culture fluid in a stepwise manner of
increasing concentrations, 5%, 10% . . .
100%. At each concentration level cell
line kinetics are measured, and growth
rates and the continued ability to produce
25 antibody are determined. At some level
(e.g., 50% ambient fluid/50% culture media)
the cells may slow their growth rate to an
unacceptable level and/or "shut-off" pro-
ducing the desired antibody. Should one or
30 both of the above occur, then the environ-
ment in vivo should be manipulated to stay
within the parameters of optimal cell
growth. This may be done by enrichment
with additional nutrients (e.g., 20% by
35 volume fetal calf serum), growth factors

1 (chemical or biological i.e., fibroblasts,
macrophages), or maintenance of proper
volume concentration, such as e.g., 45%
ambient fluid/55% culture media.

5 Completion of these steps may take several days to
several weeks depending on the cell line and the
technician.

As noted above, it has heretofore been sug-
gested that large scale production of monoclonal
10 antibodies could be effected in the amniotic fluid of
a pregnant mammal such as a cow, providing such was
accomplished prior to the onset of immunocompetency
of the fetus. However, this approach has led to a
number of problems. It has now been discovered that
15 a greatly improved method of cell growth can be pro-
vided through use of the allantoic uterine fluid, as
compared with the amnionic fluid. Use of this fluid
medium offers a number of practical advantages.
First, the allantoic fluid is more dynamically stable
20 over time in that the allantoic fluid maintains its
fluid consistency throughout the gestation period,
and does not convert into a gel-like substance which
is detrimental to cell growth and presents serious
practical problems from the standpoint of harvesting.
25 In the case of bovine allantoic fluid, there is a
steady increase in volume thereof throughout gesta-
tion, to an average volume of 6 to 9 liters, whereas
bovine amnionic fluid increases in volume up to a
gestational age of approximately 160 days, and then
30 decreases (average max. volume of 3.5 l in the bo-
vine).

In addition, cells located in allantoic
fluid are selectively isolated from both maternal and
fetal immune response. Hence, the "antigen" repre-
sented by a hybridoma cell line inoculated into the
35

1 allantoic sac is effectively separated from the
fetus, and the fetus does not ingest the allantoic
fluid as in the case of amnionic fluid. Should
"leakage" occur from the allantoic to the amnionic
5 sac, allowing an immunocompetent fetus to produce
antibody against the hybridoma, it is believed that
any antibody so produced is not secreted or excreted
by the fetus into the allantoic fluid. Inasmuch as
the amnionic sac and allantoic sac are separate
10 compartments within the uterus, bacterial contamination
will be isolated from fetal swallowing when
contained in the allantoic sac. Therefore, if infections
are detected early enough, they can be treated
with appropriate antibiotics before fetal infection
15 occurs, providing an opportunity to maintain the
pregnancy.

Use of the allantoic sac also provides a
number of surgical advantages. For example, in later
gestational ages (second-third trimester) when allantoic
20 fluid volumes begin to surpass those of the
amnionic fluid, there are resulting anatomical
changes in the position of the uterus in the peritoneal
cavity of the animal, particularly in the case
of the cow. Accordingly, this anatomical repositioning
25 allows surgical access to the uterus via flank
approaches or ventral abdominal approaches. In
contrast, one wishing to use the amnionic fluid
during the "fluid phase" (basically the first trimester)
must employ flank incisions, because ventral
30 abdominal approaches are not practical. In addition,
the fetus can be manipulated by the surgeon during
older gestational ages (6-9 months) with less danger
of inducing a spontaneous abortion. Such fetal
manipulation during the first trimester of gestation
35 in the bovine generally results in a high incidence

1 of spontaneous abortion. Finally, in the 6-9 month
gestational age, for instance, it is easy to dis-
tinguish between the yellow fluid consistency of the
allantoic fluid and the clear, contrastingly high
5 viscosity content of the amnionic sac. This makes an
easy "landmark" for the surgeon.

In terms of the nutritional qualities of
allantoic fluid, such are comparable to amnionic
fluid in many areas. For example, bovine allantoic
10 fluid has a greater content of fructose, total pro-
tein and free fatty acid, as compared to amnionic
fluid, and has somewhat less, though significant,
amounts of glucose.

For all of the foregoing reasons, then, the
15 allantoic fluid provides a more ideal environment for
cell growth, particularly in the context of produc-
tion of monoclonal antibodies. This is especially
true when cell growth occurs after the onset of
immunocompetency of the host fetus, something which
20 is precluded by prior methods seeking to employ
amnionic fluid.

EXAMPLES

The following examples described techniques
in accordance with the invention. It is to be under-
25 stood, however, that nothing in the examples should
be taken as a limitation on the overall scope of the
invention. Rather, the examples are for illustrative
purposes only, in order to elucidate the principles
of the invention.

30

EXAMPLE I

This examples sets forth a procedure for
the in vitro education of hybridoma cells. The
35 materials employed were:

POI-STOCK

Oxalacetic acid	660 mg.
Pyruvate	250 mg.
Distilled H ₂ O	50 ml.
Insulin	40 mg.

5

The above materials are slightly heated while stirring, and are aliquoted and frozen.

HT-Stock

Hypoxanthine	136 mg.
Thymidine	38.8 mg.
Distilled H ₂ O	100 ml.

15

The above materials are mixed, aliquoted and frozen.

H-T Medium

	RPMI	325 ml.
	NCTC-135	50 ml.
20	Fetal Calf Serum (FCS)	100 ML.
	POI-Stock	5 ml.
	HT-Stock	5 ml
	L-Glutamin	5 ml.
	Pen/Strep	5 ml.
25	Non-essential Amino Acids	5 ml.

20

25

30

35

The RPMI medium is commercially available (e.g., Flow Laboratories, Cat. No. 12-603) and contains inorganic salts, amino acids, vitamins and other components. Similarly, the NCTC-135 medium is available from Catalog No. 44-1100 (1980) of Gibco Laboratories, 519 Aldo Avenue, Santa Clara, California 95050. The components of the NCTC-135 medium are: inorganic salts (e.g., CaCl_2), amino acids (e.g., glycine), vitamins (e.g., niacin), co-enzymes

1 (e.g., FAD, flavin, adenine, dinucleotide), reducing
agent (e.g., ascorbic acid), nucleic acid derivative
(e.g., thymidine) and additional components (e.g.,
d-glucose).

5 The Pen/Strep is a mixture of penicillin
and streptomycin, and is available from a number of
sources, including the Pfizer Chemical Co. The pro-
duct contains 5,000 I.U./ml. penicillin and 5000
mcg./ml. streptomycin.

10

Amniotic Fluid

Obtained from cattle slaughterhouse from
pregnant cow at not more than 80 days gestation. The
fluid is filtered through Seitz filters (several
15 steps) beginning with a pore size of 1.0 m, and
decreasing pore size in stages (0.5, 0.2 and 0.1
mm). The filtered fluid is then heated to 56 degrees
centigrade for 30 minutes. The fluid may then be
tested for bovine virus diarrhea, bluetongue, lepto-
20 spirosis, mycoplasma, or any other agents deemed
necessary by the investigation laboratory, for ex-
ample BVD, lepto, bluetongue, the presence of endo-
toxin, bacteria, etc.

25 Heat Inactivated FCS

FCS is heated to 56 degrees C for 30
minutes, and is filtered before use using staged
Seitz filters having pore sizes of 0.5 and 0.1 m.

In a specific cell education procedure, the
30 cells (produced by fusion of NS-1 myeloma cells with
mouse spleen cells immunized against IBR in cattle)
were initially cultured at 38.3-39 degrees centigrade
in H-T medium to a density of 10^5 cells/ml. Ten per-
cent heat inactivated FCS and five percent amniotic
35 fluid were then added, and the mixture was allowed to

- 27

1 incubate at 38.3-39 degrees centigrade for 48 hours.
At the end of this period, another 5% amniotic fluid
was added, and incubation was allowed to continue at
the noted temperature.

5 When the cells grew to a density of 10^6
cells/ml., the cells were split 1:3 using H-T medium
with ten percent heat inactivated FCS and twenty per-
cent amniotic fluid. The cells were then allowed to
grow to 10^6 cells/ml. density at 38.3-39 degrees
10 centigrade, and were again split using the same pro-
cedure but with thirty percent amniotic fluid. Fin-
ally, all of the resultant cell colonies were grown
to a density of 10^6 cells/ml., and were checked for
antibody production.

15 The entire cell education procedure took
four days.

EXAMPLE II

20 In this in vitro test, hybridoma cells as
used in Example 1 were employed.

In a control, the cells were removed from
their standard media and placed in uterine fluids
(amniotic and allantoic). A death rate of 90-100
25 percent occurred within 24 hours of incubation at 39
degrees centigrade. No cells were found alive or
producing antibody after 72 hours.

In a second test, the protocol above was
generally followed except that the cells and their
standard culture media (RPMI) was added to the uter-
ine fluids (resultant media was 70 percent RPMI, 30
percent uterine fluid). The initial death rate was
30 decreased to 60 percent after 24 hours of incubation
at 39 degrees centigrade. The cells continued to
multiply in vitro with the 70 percent RPMI/30 percent
35

1 uterine fluid media during standard hybridoma culture
and feeding techniques.

The second test was repeated except that
FCS was added stepwise in various concentrations (5%;
5 10%; 15%; and 20%). These combinations were success-
ful in lowering the initial death rate to 45-50 per-
cent under standard in vitro culture methods at 39
degrees centigrade. Over several weeks of culturing,
the proportion of RPMI was gradually reduced to less
10 than 10 percent. At this point the hybridomas were
educated, and were placed in the appropriate (90%
amniotic fluid/10% FCS) uterine fluids (in vitro) for
multiplication.

Next the cells were fed 10 percent FCS by
15 volume every forty-eight hours for six feedings. At
this point the cells continued to grow (multiply) at
their normal rate plus maintained their antibody pro-
duction level, even though subsequent feedings were
discontinued. The first decline in multiplication
20 rate was observed twelve days after the last feeding.
Normally, hybridomas must be fed every forty-eight
hours; however, the described education to the amni-
otic fluid allowed this time period to be extended.

25 EXAMPLE III

This example gives a procedure for the
education of myeloma or hybridoma cells prior to
inoculation into the amniotic fluid of a pregnant
cow.

30 Materials

1. Allantoic Fluid

Obtained from cattle slaughterhouse from
pregnant cows at 3-9 months gestation. The fluid is
clarified by low speed centrifugation and sterilized
35 by filtration successively through a series of fil-

1 ters from 1.0 m down to 0.2 m. Endotoxin-free
 samples of fluid are pooled for use in tissue cul-
 ture.

2. Media

5 Dulbecco's Minimal
 Essential Medium 40-75%

 Fetal calf serum 20

 Pen/Strep, 10,000 units/
 10,000 mcg 1

10 Nutrient supplement¹ 4

 Allantoic fluid 0-40

¹Nutrient supplement
 L-glutamine, 200,mM, 100X

15 MEM Amino Acids, 100X

 MEM Non-essential Amino
 Acids, 100X

 Vitamins, 100X

Method

20 In a control test, myeloma cells capable of
 producing antibody against surface antigens of pneu-
 mococcus organism were placed in allantoic fluid and
 tested for survival over a period of 72 hours. A
 death rate of 50% occurred in 24 hours and at 72
 25 hours 10 percent of the cells were viable.

 These myeloma cells were used in an in
vitro education test to allantoic fluid. Cells were
 seeded at 5×10^5 /ml in complete medium. Subsequent
 passage was set up with the media supplemented by 10%
 30 every 48 hours during feeding, up to a maximum level
 40 percent allantoic fluid. During this test the
 growth rate of the myeloma cells remained steady up
 to the 40 percent allantoic fluid level.

EXAMPLE IV

35 The cell line chosen for this study was the

1 murine BALB/C myeloma MOPC 315J which constitutively
produces a monoclonal IGA molecule having binding
specificity for the trinitrophenol group (TNP). 315J
cells are grown in Dulbecco's minimal essential
5 medium supplemented with 20 percent fetal calf serum,
antibiotics and a nutrient cocktail consisting of
L-glutamine, MEM amino acids, MEM non-essential amino
acids, sodium pyruvate, and vitamins. Supernatants
of 315J cells grown for a period of time routinely
10 contain up to 1 microgram/ml IgA as measured by
radio-immunoassay (RIA). Cultures are routinely
grown from 5×10^5 cells/ml to a concentration $2-5 \times 10^6$
cells per ml. with refeeding every 2 days.
Frozen stocks are maintained at -70 degrees centi-
15 grade or in liquid nitrogen.

The antibody production by 315 J cells is
routinely measured by RIA or any enzyme-linked immun-
oadsorbant assay (ELISA). These assays will quanti-
tate the concentration of antibody in either culture
20 fluids or uterine fluids. The rosette assay will
identify and quantitate the number of 315J myeloma
cells in culture or from uterine fluids. This assay
involves attaching the binding antigen (TNP) to sheep
red blood cells. Using these assays (RIA, ELISA AND
25 Rosette) both myeloma (hybridoma) cells and their
products, monoclonal antibodies, can be identified
and quantitated.

Respective colonies of cells were placed in
allantoic fluid, in the usual complete medium, and in
30 balanced salt solution and incubated in vitro. Tests
were made for cell survival over a period of 72
hours. A death rate of fifty percent occurred in 24
hours and at 72 hours 10 percent of the myeloma cells
were viable when they were in allantoic fluid. On
35 the other hand, the cells in balanced salt solution

1 died at a faster rate, 50 percent in 6 hours and 100
percent in 20 hours. Myeloma cells cultured in
complete media doubled in number by 24 hours. These
5 results demonstrated that the allantoic fluid is not
toxic but does not have enough nutrients to induce
these cells to divide.

The next step was to educate the myeloma
cells to grow in medium containing allantoic fluid.
Cells were seeded at 5×10^5 /ml in complete medium.
10 Subsequent passage was set up with the media supplemented
with 10 percent allantoic fluid. The percentage
of allantoic fluid was increased by 10 percent
with every 48 hours feeding up to 40 percent
allantoic fluid. By this method the growth rate of
15 the myeloma cells remained steady.

Myeloma cells, both educated and uneducated,
were then injected in the allantoic fluid of
pregnant sheep at approximately 100 days gestation,
after the onset of fetal immunocompetency. The
20 device employed for the cell inoculation and subsequent
fluid withdrawals was of the type illustrated
in Figs. 10-11, and the device was installed in the
manner described previously, where the inner end of
the fluid conduit was affixed to the allantoic sac of
25 the sheep. Pathological samples of tissue from the
sheep indicated that myeloma-like cells are found in
groups in the placental tissue in cases of both
educated and uneducated myeloma cell injection.

Cells taken from samples of sheep allantoic
fluid were also counted with time after injection.
30 In one example, 10^8 uneducated myeloma cells were
injected into a sheep with 500 ml. allantoic fluid
(estimated). Ten ml. samples were removed daily for
7 days. Beginning with day 1, 8.5×10^7 cells were
35 estimated to be in the allantoic fluid. Cell numbers

0103081

-32-

1 decreased daily but by 7 days 2.5×10^7 cells were
still present. This is a tremendous number of cells
to be left when the cells injected were uneducated
and no nutrients were added for their growth.

5

10

15

20

25

30

35

1 Claims

5 1. Apparatus for selectively withdrawing fluids from, or adding fluids to, and animal's body, said apparatus comprising:

10 an assembly including a tubular, fluid-conveying element, and means operably coupled with said element for normally blocking fluid flow therethrough and being openable for selectively permitting fluid flow through said element;

15 means for operatively securing said assembly to said animal's body with the outer end of said element being adjacent the exterior surface of the animal's skin;

20 an elongated, flexible, fluid-conveying conduit having an inner end and an outer end, said outer end being operably coupled and in fluid flow communication with said element when said assembly is operatively secured to said animal, said inner conduit end being configured for insertion through the wall of a specific organ or the like of said animal, with the innermost portion of the flexible conduit lying within the organ or the like,

25 said conduit being of a length greater than the shortest distance between said element and the point of insertion thereof through the wall of said organ or the like for, in conjunction with the flexibility of the conduit, permitting and accommodating natural movement of said organ or the like over time; and

30 35

1 means for physically attaching and interlocking
said inner end of said conduit to said wall
of said organ or the like with said inner-
most portion lying within the organ or the
5 like, including structure for preventing
leakage of fluid from said organ or the
like.

2. Apparatus as set forth in Claim 1,
10 including selectively operable valve means within
said assembly.

3. Apparatus as set forth in Claim 2,
said valve means including:
15 a flow blocking plug located within said ele-
ment;
structure defining a wall surface adjacent said
plug and complementary with the plug;
means for biasing said plug against said wall
20 surface for blocking fluid flow past the
plug; and
means engageable with the plug for selectively
shifting the plug away from said wall sur-
face, and for permitting fluid flow through
25 said element and past said plug when the
plug is in said shifted away position.

4. Apparatus as set forth in Claim 1,
said innermost portion of said conduit including a
30 plurality of apertures therethrough.

5. Apparatus as set forth in Claim 1,
said attaching and interlocking means comprising a
support collar disposed about said conduit inter-
35 mediate the ends thereof.

1 6. Apparatus as set forth in Claim 5,
said support collar being suturable for attachment
and interlocking to said wall of said organ or the
like.

5 7. Apparatus as set forth in Claim 5,
said collar being perforate.

10 8. Apparatus as set forth in Claim 5,
said collar being flexible.

15 9. Apparatus as set forth in Claim 5,
said collar extending at least about one-quarter of
an inch outwardly from said conduit.

20 10. Apparatus as set forth in Claim 1,
said innermost portion of said conduit being of a
different diameter than the remainder of said con-
duit.

25 11. Apparatus as set forth in claim 10,
said innermost portion having a diameter greater than
that of the remainder of said conduit.

30 12. Apparatus as set forth in claim 1, said
conduit comprising, for at least a segment of the
length thereof, a pair of telescopically interfitted
tubular components.

35 13. Apparatus as set forth in Claim 1,
said assembly-securing means comprising an outer
tubular component receiving said tubular element, and
an external screw thread about said tubular compo-
nent.

35

1 14. A method of periodically and select-
ively removing fluids from, or adding fluids to, a
specific organ or the like of a living animal said
method comprising the steps of:

5 providing a tubular, fluid-conveying element;
operatively securing said element to said
animal's body with the outer end of the
element adjacent the exterior surface of
the animal's skin;

10 operatively coupling an elongated, flexible,
fluid-conveying conduit to, respectively,
said element and said organ or the like,
said coupling step including the steps of making
an incision in the wall of said organ or
15 the like and inserting the inner end of
said conduit into said organ or the like
until the innermost portion of the conduit
lies within the organ or the like;

20 said conduit being of a length greater than the
shortest distance between said element and
said incision for, in conjunction with the
flexibility of the conduit, permitting and
accommodating natural movement of said
organ or the like over time;

25 physically attaching and interlocking said inner
end of said conduit to said wall of said
organ or the like, with said innermost
portion lying within the organ or the like,
for preventing leakage of fluid from said
30 organ or the like; and

periodically and selectively withdrawing fluid
from, or adding fluid to, said organ or the
like by conveying fluid through said con-
duit and element.

1 15. The method of Claim 14, said organ or
the like being a uterus.

5 16. The method of Claim 15, including the
step of inserting said inner end of the conduit into
the allantois.

10 17. A method of growing cells comprising
the steps of:

 selecting a body cavity of a living animal as a
 situs for cell growth;
 obtaining ambient fluid characteristic of said
 body cavity;
15 educating said cells by contacting the cells
 with said ambient fluid, in vitro;
 innoculating said educated cells into said
 selected cavity containing said ambient
 fluid;
20 allowing said cells to multiply in said ambient
 fluid; and
 harversting said cells or the secretory products
 of said cells.

25 18. The method of Claim 17, said educating
step comprising the steps of repeatedly contacting
said cells with respective quantities of said ambient
fluid over a period of time.

30 19. The method of Claim 18, said quanti-
ties increasing in volume over said period of time.

35 20. The method of Claim 17, said ambient
fluid being amnionic fluid from the uterus of a preg-
nant host.

1 21. The method of Claim 17, said cavity
being a uterus of a pregnant host, said ambient fluid
having uterine fluid of said host, said host being an
animal wherein the animal's placenta serves as a
5 barrier to the passage of maternal antibodies into
the uterus.

22. The method of Claim 21, said host
being selected from the group consisting of sheep,
10 horses, pigs, and cows, said cavity being the uterus.

23. The method of Claim 17, said cells
being maintained at a temperature of about 38.3-39
degrees centigrade during said educating step.
15

24. The method of Claim 17, said ambient
fluid being derived from said host.

25. The method of Claim 17, said ambient
20 fluid being obtained from the same type of animal as
said host.

26. The method of Claim 17, said educating
step comprising the steps of:
25 contacting said cells with a fluid containing from
about 5 to 10% by volume of said ambient
fluid, and allowing said cells to cultivate
therein; and
thereafter contacting said cells in a stepwise
30 manner with respective fluid samples con-
taining increasing quantities of said ambient
fluid.

1 27. The method of Claim 17, said cells being
selected from the group consisting of hybridoma cells
or any cell line producing a biologically active pro-
tein or product.

5 28. A method of in vitro cell growth com-
prising contacting cells to be grown with the uterine
fluid of a pregnant animal under conditions whereby
said cells will multiply, and allowing said cells to
10 grow therein.

 29. The method of Claim 27, said uterine
fluid being allantoic fluid.

15 30. The method of Claim 27, including the
step of repeatedly contacting said cells with respec-
tive fluids containing increasing concentration of said
uterine fluid over a period of time.

20 31. A method of growing cells, comprising
the steps of:

 inoculating said cells into the allantois of a
pregnant host;

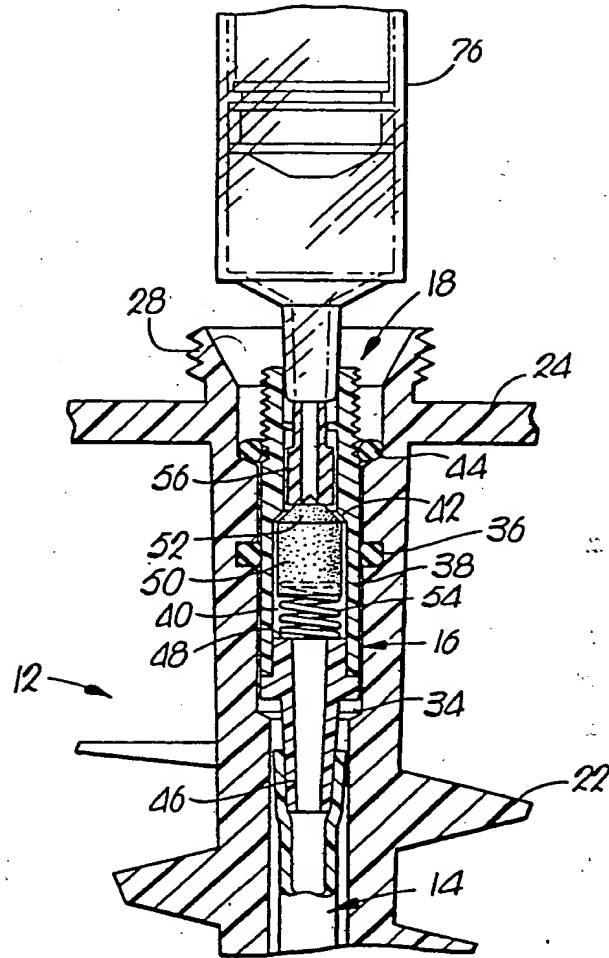
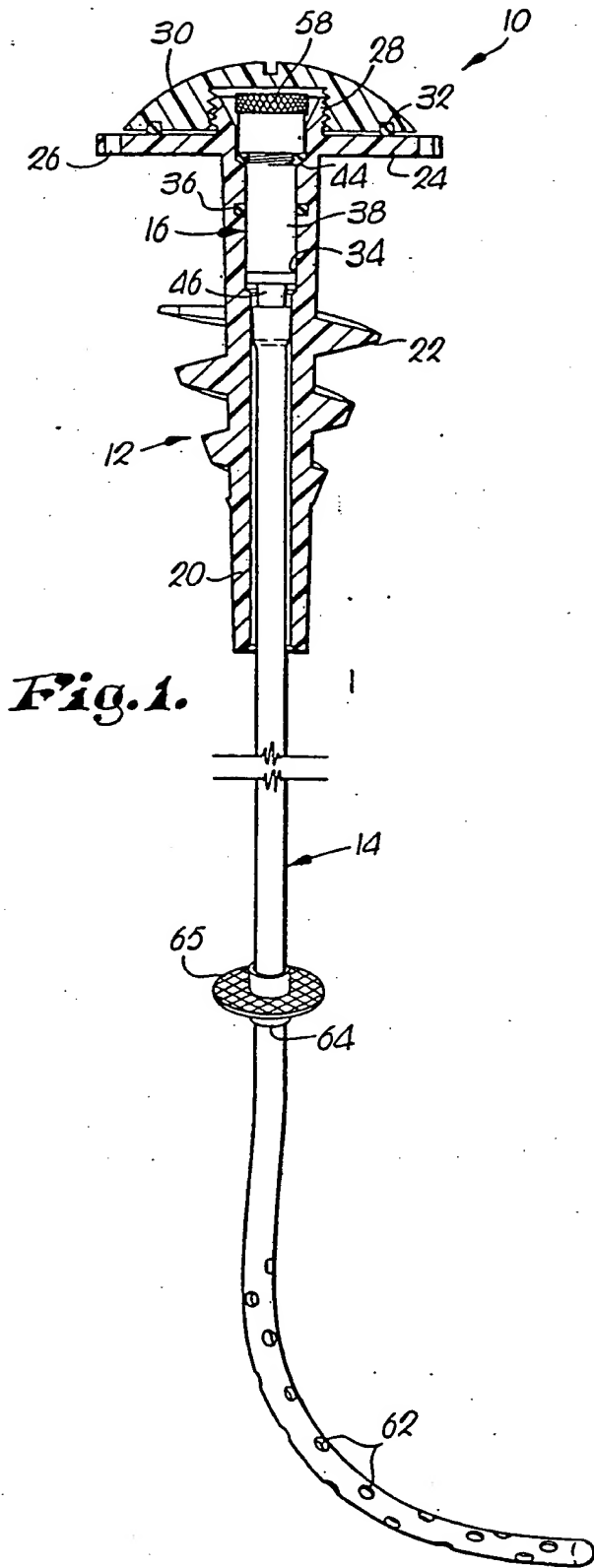
 causing said cells to multiply in said allantois
25 over a period of time,

 at least a portion of said time period being
after the onset of imunocompetency of the
host's fetus; and

 thereafter harvesting said cells or the secre-
30 tory products thereof.

 32. The method of Claim 31, said host
being selected from the group consisting of sheep,
horses, pigs, and cows.

1/4



1 33. The method of Claim 31, including the
step of, prior to said inoculation, contacting said
cells in vitro with a quantity of allantoic fluid
from the host or from the same type of animal as the
5 host.

 34. The method of Claim 31, said in vitro
contacting step comprising the steps of repeatedly
contacting said cells with respective fluid samples
10 containing increasing concentrations of said allantoic fluid.

 35. The method of Claim 31, said inoculation being carried out after the onset of immunocompetency of said fetus.
15

 36. The method of Claim 31, including the
step of adding nutrients and/or growth factors for
said cells to the allantoic during said time period.
20

 37. The method of Claim 31, said host
being a cow.
25

30

35

40

3/4

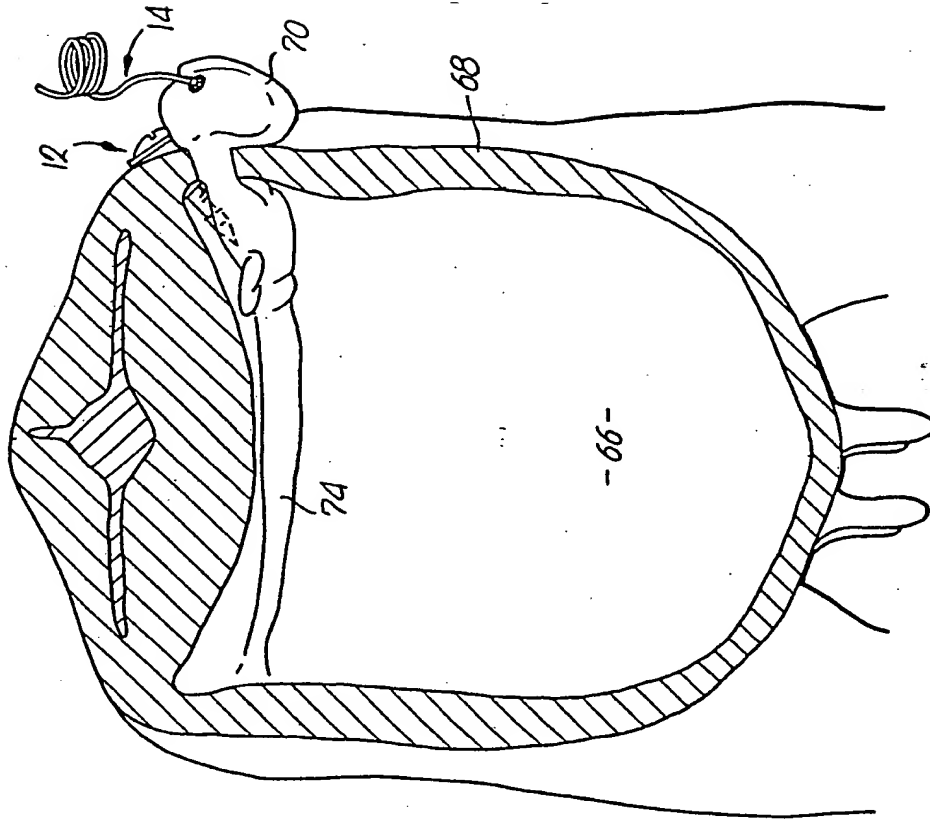


Fig. 8.

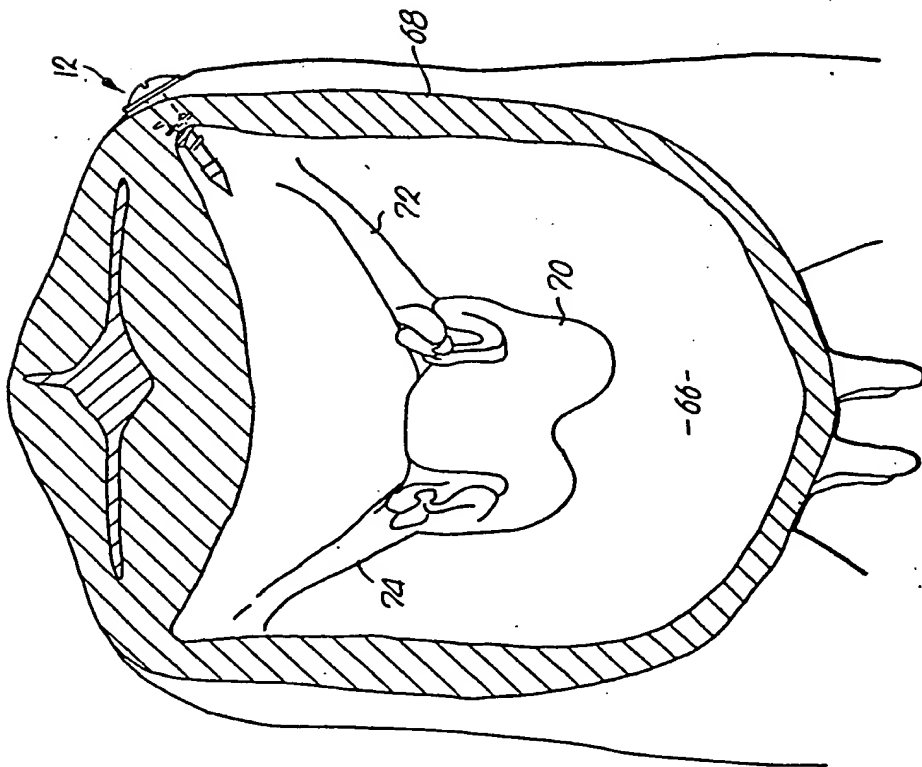


Fig. 9.

2/4

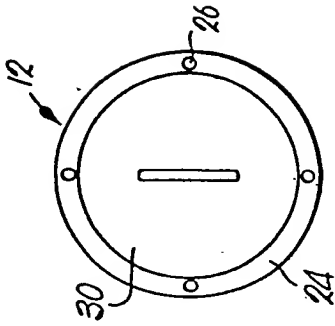


Fig. 4.

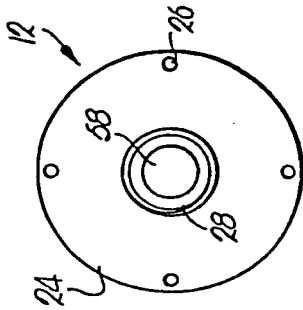


Fig. 5.

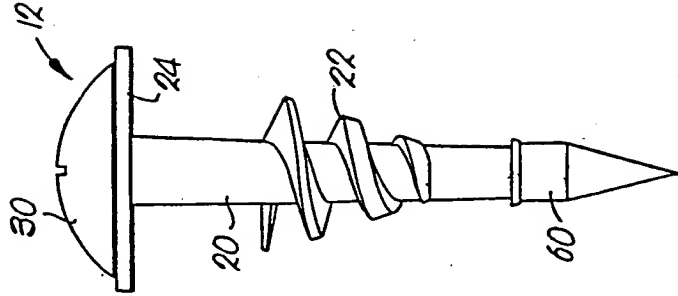


Fig. 3.

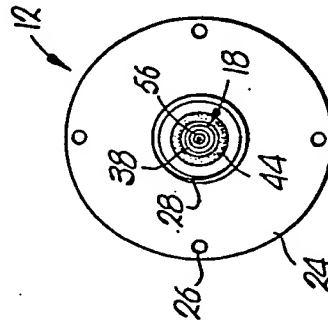


Fig. 6.

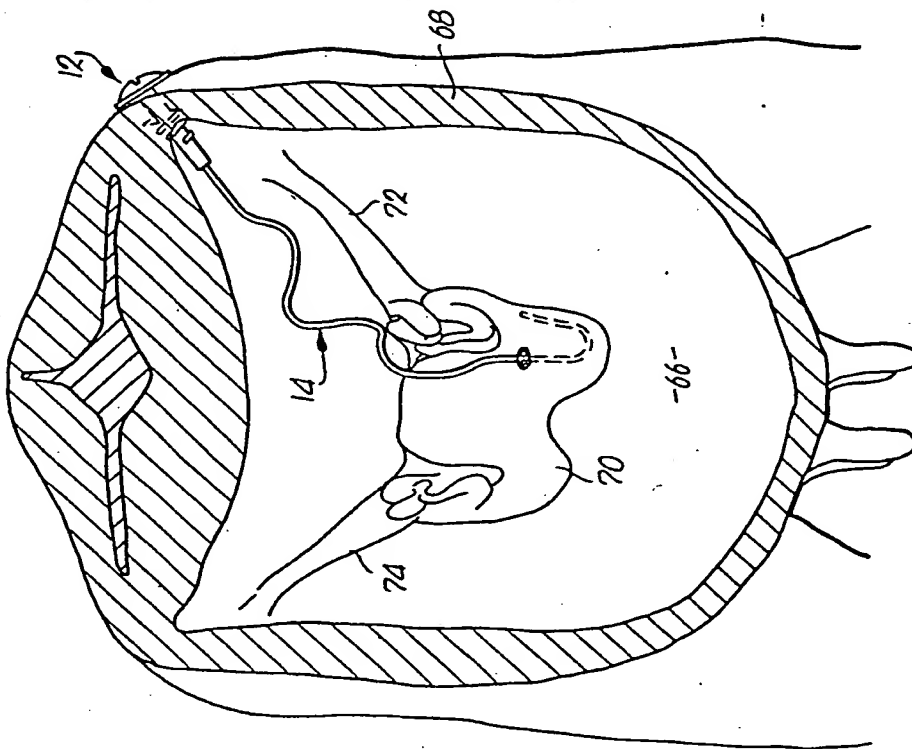


Fig. 9.

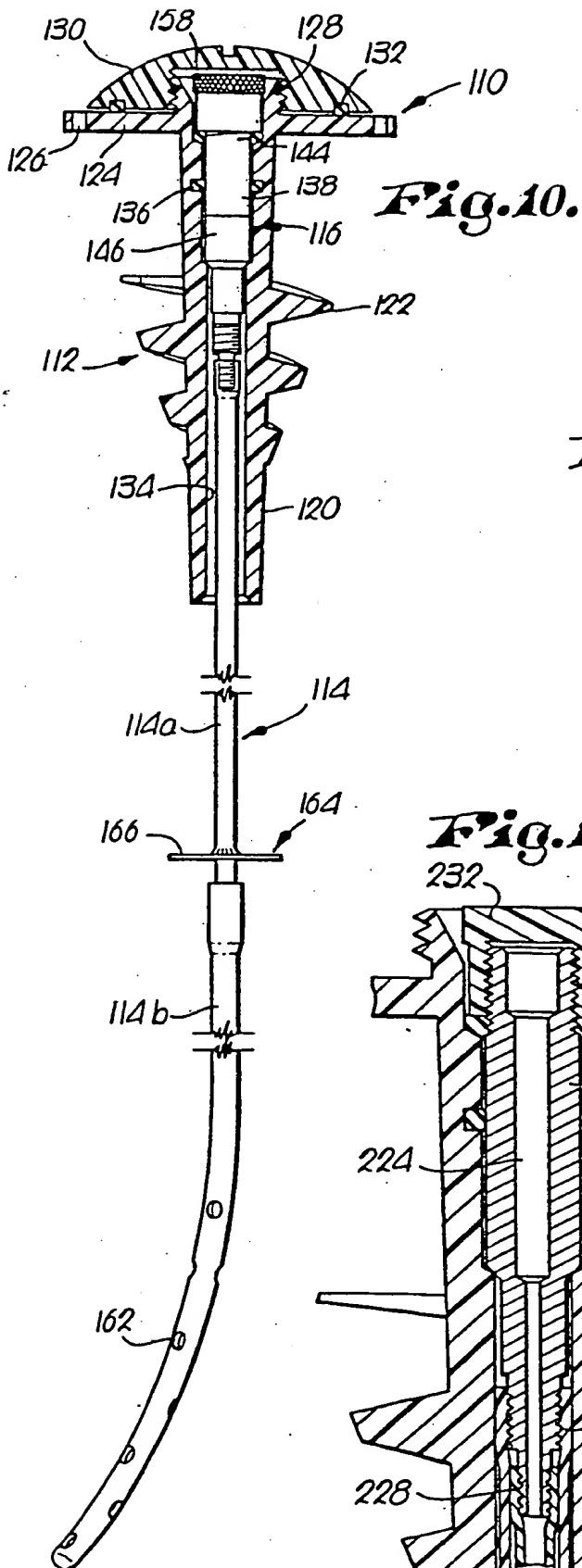


Fig. 11.

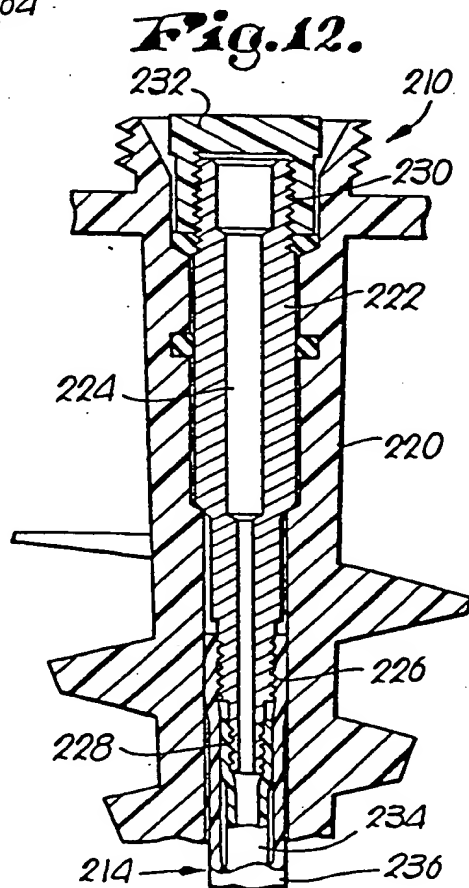
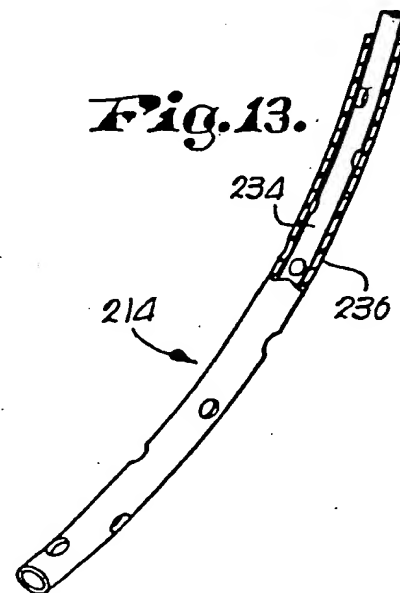


Fig. 13.



THIS PAGE BLANK (USPTO)